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Award Number: DAMD17-99-1-9520

TITLE: The Contributions of 8p Loss and 8p Gain to the Malignant Phenotype in Human Prostate Tumors

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REPORT DATE: September 2000

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2000	Annual Summary (1 Sep 99 - 31 Aug 00)

4. TITLE AND SUBTITLE
The Contributions of 8p Loss and 8p Gain to the Malignant Phenotype in Human Prostate Tumors

5. FUNDING NUMBERS
DAMD17-99-1-9520

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
This report contains colored photos

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12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

14. SUBJECT TERMS
Prostate Cancer

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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The three specific aims of my project are:

- *Specific Aim 1:* transfect normal prostatic epithelium with the E6 and E7 genes of HPV16 to produce immortalized cell lines, then genotype these cell lines to determine 8p and 8q status: retention of 8p sequences, loss of 8p sequences, or loss of 8p+gain of 8q sequences [iso(8q)];

- *Specific Aim 2*: determine whether loss of 8p sequences or loss of 8p+gain of 8q sequences is associated with expression of the transformed or invasive/metastatic phenotypes in the E6/E7 immortalized cells;

- *Specific Aim 3 (Long Term Goals):* isolate 8p-specific and 8q-specific genes that contribute to the transformed or invasive/metastatic phenotype in E6/E7 immortalized cells.

After joining Dr. Macoska's laboratory, I have learned basic cancer biology and the techniques used for the project. I attend weekly journal club meetings organized by my mentor Dr. Macoska. I also attend seminars in cancer biology organized by Hematology/Oncology division, comprehensive cancer center, and other various departments of the University. These seminars are organized on a weekly basis.

For my project, I have been growing explant cultures of primary prostate cells from the tissue samples procured from our collaborator, Dr. Mark Rubin (Department of Pathology). Initially, I grew cells in defined Keratinocyte growth medium (KGM)

containing 5% bovine fetal serum on collagen coated plates. These efforts were not successful , so the concentration of serum was increased to 20%. The cells grew better in KGM with 20% FBS but, upon trypsinization for passaging, these cells became senescent and died. Dr. Macoska then suggested a shift to another defined medium (5% HIEC i.e. Ham's F12 supplimented with 5% fetal bovine serum, hydrocortisone, insulin, epidermal growth factor and cholera toxin) in which good cells growth was observed. The cells were grown on uncoated plates to facilitate trypsinisation.

After growing the cells and successfully passaging them, I have immortalized these cells using a recombinant replication deficient retrovirus carrying E6 and E7 genes of HPV-16, supplied by our collaborator Dr. Johng Rhim. A number of cultures were immortalized in this way.

To date, I have successfully immortalized cells from five tissue samples designated as N1, N10, N12, N15 and N17. Immortalized cells from N1 sample are now at the 45 passage level, while control normal cells from the same sample are growing very slowly at 34 passage. Cells from the other immortalized samples are growing at tenth passage level, while normal control cells from respective samples senesced and died after third passage.

CELL LINE	PASSAGE
N1	45
N10	10
N12	10
N15	10
N17	10

Photographs of four of these five cell lines are shown below in Figure 1:

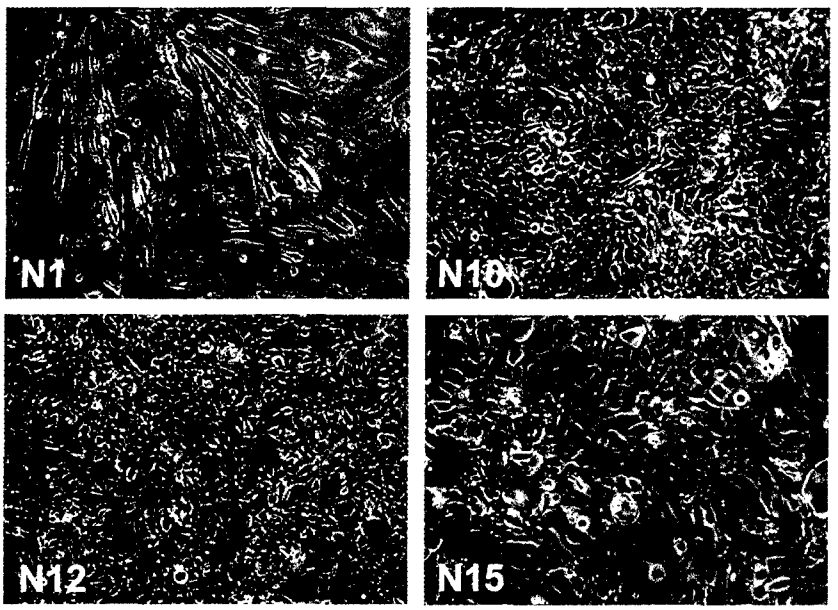

Figure 1. **Photomicrographs of four cell lines established from normal primary prostate cells.** N1 is fibroblastic in cell type, whereas N10, N12 and N15 are mixed basal and luminal epithelial cells types

Epithelial-derived cell lines. To check the nature of the tissue i.e. normal, PIN or cancerous before putting in culture, a small sample was taken for histological examination. For N10, N12, N15 and N17, the tissue was predominantly epithelial. Immunohistochemical analysis and subcloning experiments are currently underway to separate basal and luminal epithelial cell types and establish cell type subclones of these cell lines.

Stromal-derived cell lines. The tissue in the case of the N1 sample was predominantly stromal, i.e., fibroblastic cells. The N1 cells were also examined immunohistochemically using antibodies against cytokeratins, vimentin and factor VIII like antigen. These cells stained strongly with anti vimentin antibody confirming that these cells were in fact fibroblastic in nature (specific aim 1). Presently N1 cells are also being compared with other established cell lines for androgen response *in vitro*. The

data is being analysed (specific aim 3). N1 cells immortalized with HPV 16 retrovirus has also been assessed cytogenetically using spectral karyotyping (specific aim 2). As seen in Figure 2, the N1 karyotype is completely normal.

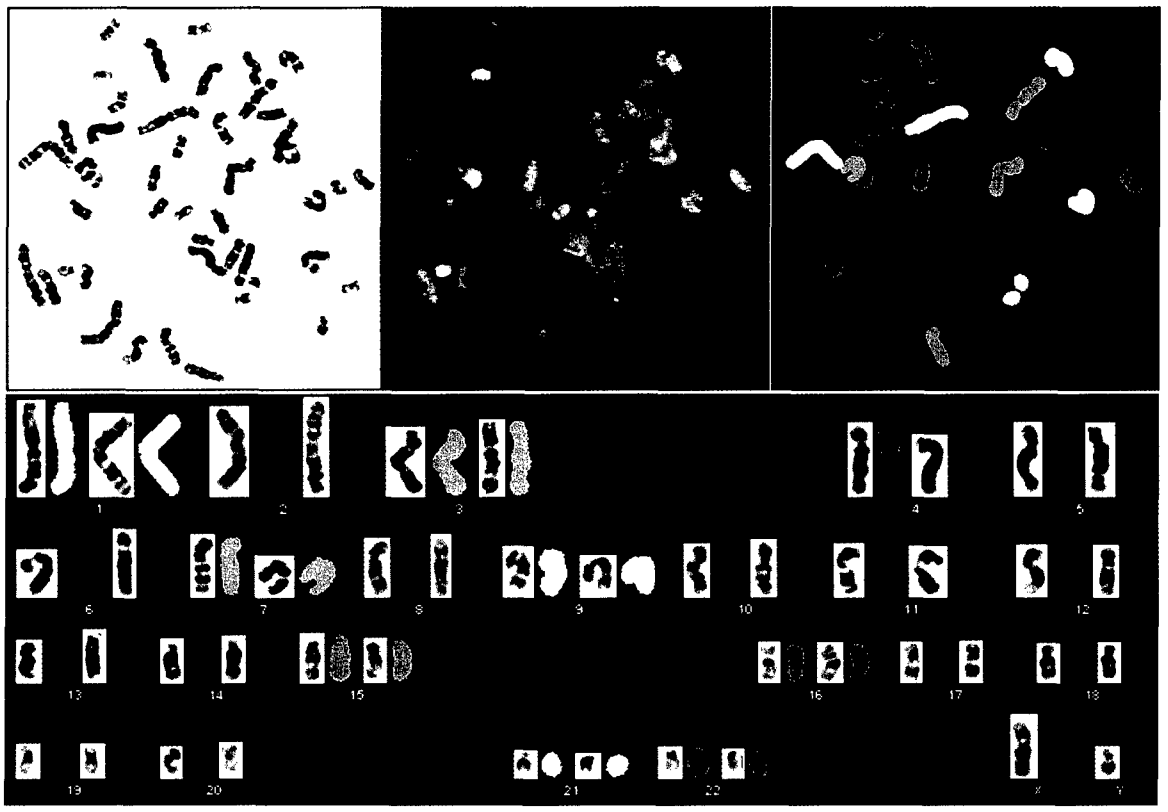

Figure 2. **Spectral Karyotype Composite of the N1 Cell Line.** Upper Panel: G-banded preparation of metaphase chromosomes from N1 cells (left), hybridized to SKY paints (middle), and after pseudo-color application (right), as described in the text. Lower Panel: Composite karyotype showing G-banded and pseudo-colored chromosomes. The karyotype for the cell shown is: 46, XY.

Simultaneously, I also tried to transfect plasmid containing large T antigen gene of SV40 virus into primary prostate cells. I used two constructs one simple plasmid pMT 10D with no selection option, and another plasmid, pSVT-CMV, that can be selected for geneticin resistance. A number of tranfection agents have been tried (viz lipofectamine, genejammer, Calcium phosphate, gene factor and fugene, etc.). The most suited for the prostate primary cells are genefactor and lipofectamine. These two agents give ~7%

transfection efficiency but on G 418 selection all the cells died. We are presently cloning the SV 40 large T antigen gene into a bicistronic retroviral vector to facilitate the introduction and expression of large T antigen gene in primary prostate cells.

In summary the specific aim number one has been achieved partially, as I have now established five immortalized cell lines which are currently undergoing cell type characterization and genetic evaluation. I am continuing to establish cell lines and will attempt transformation with SV40 Large T in order to induce genetic changes, e.g., 8p loss and/or 8q gain. Homogenous subclones of all cell lines will established and characterized immunohistochemically to determine cell type. Later passage cells will be spectrally karyotyped to established 8p and 8q status (specific aim 2), then evaluated for expression of the malignant phenotype (specific aim 3).

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